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Aspirin induces alterations in low-density lipoprotein and decreases its catabolism by cultured human fibroblasts

C. Mazière, S. Goldstein, M. Moreau, J.C. Mazière and J. Polonovski

Laboratoire de Biochimie et CNRS UA 524, Faculté de Médecine Saint-Antoine, 27 rue Chaligny, 75012 Paris, France

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Aspirin interacts in vitro with human low-density lipoprotein (LDL), which results in a decrease in free amino groups of apolipoprotein B and an increase of electrophoretic mobility of the particle. The aspirin-treated LDL was less efficiently recognized than native LDL by the apo B/E receptor of fibroblasts. These results suggest that aspirin in long-term treatment could influence the LDL-receptor pathway. However, aspirin-treated LDL did not bind to the scavenger receptor of macrophages.

Aspirin; LDL modification; Fibroblast receptor

1. INTRODUCTION

It has been reported that human serum albumin and other serum proteins such as immunoglobulins, transferrin and fibrinogen are altered after exposure to therapeutic concentrations of acetylsalicylic acid (ASA, aspirin), either in vitro or in vivo [1,2]. These alterations arise from acetylation of lysine residues [3]. It is also known that chemical modification such as acetylation of the lysine residues of low density lipoproteins (LDL) decreases their binding, internalization and degradation by fibroblasts [4]. The catabolism of the modified LDL is shifted to the 'scavenger receptors' of macrophages, which accumulate high amounts of cholesteryl esters; this process results in foam cell formation [5,6] and subsequent development of atherosclerotic lesions.

The aim of the present work was to study the effects of therapeutic doses of ASA upon LDL and to determine to what extent ASA may alter LDL binding, internalization and degradation by cultured human fibroblasts. As altered LDL can be recognized by macrophages when the negative net charge of the particle is sufficiently increased [7], we also tested ASA-LDL on J774 mouse macrophages.

Correspondence address: C. Mazière, Laboratoire de Biochimie et CNRS UA 524, Faculté de Médecine Saint-Antoine, 27 rue Chaligny, 75012 Paris, France

2. MATERIALS AND METHODS

2.1. *Materials*

$Na^{125}I$ (13–17 Ci/mg) was from Amersham (England) and Dulbecco's modified minimum essential medium (MEM) with Earle's salts and fetal calf serum from Gibco (Grand Island, NY). MRC5 human fetal lung fibroblasts were purchased from BioMérieux, France and J774 mouse macrophages from the American Type Culture Collection (Rockville, MD). Ultroser G was from Industries Biologiques Françaises (France). Silica gel plates F 1500 were from Schleicher and Schuell (Dassel, FRG).

2.2. *Cell culture*

Cells were cultured in 35 mm Nunc petri dishes containing 2 ml Dulbecco's MEM medium supplemented with 20 mM Hepes buffer (pH 7.4), 100 U/ml penicillin, 100 μg/ml streptomycin and 10% (v/v) fetal calf serum, at 37°C in a humidified atmosphere of 5% CO_2. Experiments were per-

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formed on confluent cells, approx. 7 days after seeding.

2.3. *LDL preparation and labeling*

LDL was prepared from normal human serum by 3-step ultracentrifugation at 105000 × *g* in a Beckman L5-50 instrument, according to Havel et al. [8]. The LDL was taken as the 1.024–1.050 fraction. Labeling was performed as described by Bilheimer et al. [9]. Protein determination was done according to Lowry et al. [10]. The specific activity was about 250–350 dpm/ng LDL protein, and free [^{125}I]iodine in the preparation was always below 2.5%.

2.4. *LDL modification and characterization*

Acetylated LDL (AC-LDL) was prepared using the technique of Basu et al. [11]. For modification by ASA (ASA-LDL), LDL (1.5 mg) was incubated with 3 mg ASA in 0.1 M phosphate buffer (pH 7.4) at 37°C for 24 h, according to Hawkins et al. [2]. After incubation, the LDL was extensively dialysed against 0.1 M phosphate buffer (pH 7.4). The product was chromatographed on Sephadex G-75, using 0.025 M Tris, 0.0001% EDTA (pH 7.6) as elution buffer. The extent of derivatization of lysine residues in modified LDL was estimated by the colorimetric trinitrobenzenesulfonic acid (TNBS) assay for primary amines [12], using HCl-lysine as standard. Electrophoresis was performed on universal electrophoresis agarose film (Corning Chemicals, Palo Alto, CA) and stained with oil red O. LDL and the modified products were subjected to SDS-polyacrylamide gel electrophoresis (Weber and Osborn) in 4% flat gels. Double immunodiffusion using a rabbit antiserum to LDL was performed under the conditions in [13].

2.5. *LDL binding, internalization and degradation by cultured human fibroblasts*

Binding, internalization and degradation of ^{125}I-LDL by human fibroblasts and ^{125}I-AC-LDL by J774 mouse macrophages were studied as described in [14]. For maximal expression of LDL receptors, fibroblasts were preincubated for 24 h in medium devoid of lipoproteins (Dulbecco's MEM + 2% Ultroser G). Cells were incubated in the presence of 10 μg/ml ^{125}I-LDL (fibroblasts) or ^{125}I-AC-LDL (macrophages), in the absence or presence of increasing concentrations of unlabeled ASA-LDL, AC-LDL or native LDL. Results are expressed as percentages of controls.

3. RESULTS AND DISCUSSION

ASA-modified LDL submitted to Sephadex G-75 chromatography eluted in a peak at the void volume (coincident with Blue dextran 2000) and presented a small shoulder. A similar profile, indicating slight heterogeneity of particles, was observed for native LDL and AC-LDL (not shown). For all experiments, only fractions corresponding to the major peak were used.

Derivatization of the lysine residues as assessed by TNBS assay was about 9% in ASA-LDL and about 53% in AC-LDL.

Fig.1a shows that the electrophoretic mobility of ASA-LDL was increased by 2.4 ± 1.6 mm (n = 9) as compared to native LDL. This indicates a higher negative net charge of the particle. In comparison, AC-LDL mobility was increased by 23 ± 2.5 mm (n = 10).

Polyacrylamide gel electrophoresis did not show any alteration in ASA-LDL as compared to LDL, whereas AC-LDL presented some discrete aggregation. Upon immunodiffusion, when compared to native LDL, ASA-LDL and AC-LDL showed patterns of identity and partial identity, respectively (fig.1b). This indicates a larger modification of the antigenic sites in AC-LDL than in ASA-LDL.

Competition studies performed on cultured

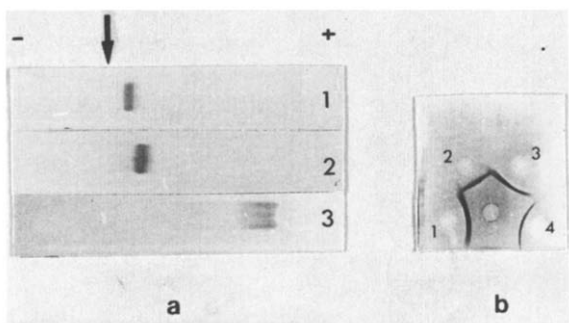

Fig.1. (a) Agarose gel electrophoresis of native and modified LDL: 1, native LDL; 2, ASA-LDL; 3, AC-LDL. The arrow indicates the origin. (b) Immunodiffusion pattern of LDL, AC-LDL and ASA-LDL reacting with an antiserum to LDL. Central well, antiserum to LDL. Peripheral wells: 1, 3, LDL; 2, AC-LDL; 4, ASA-LDL. Soudan black staining.

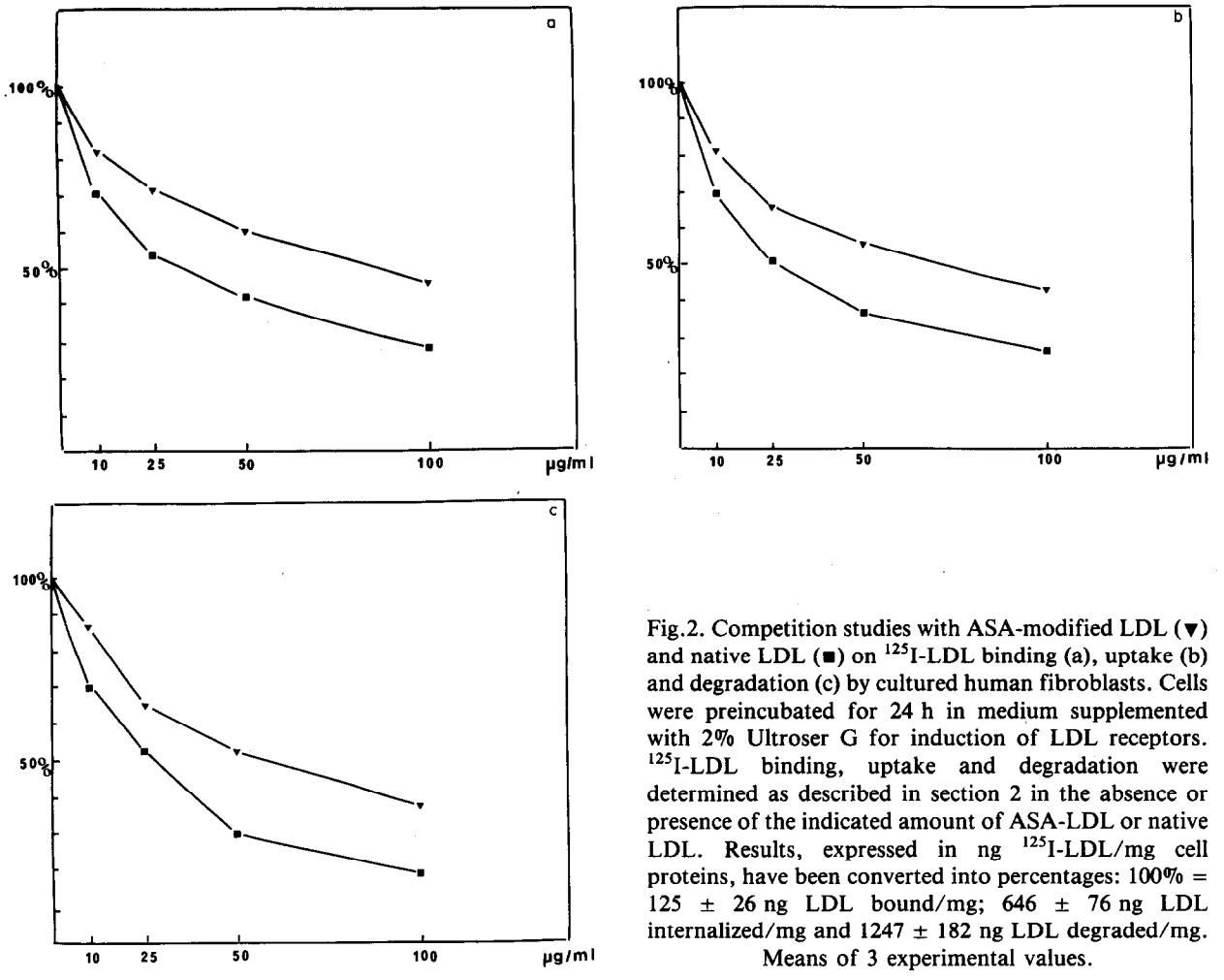

Fig.2. Competition studies with ASA-modified LDL (▼) and native LDL (■) on ^{125}I-LDL binding (a), uptake (b) and degradation (c) by cultured human fibroblasts. Cells were preincubated for 24 h in medium supplemented with 2% Ultroser G for induction of LDL receptors. ^{125}I-LDL binding, uptake and degradation were determined as described in section 2 in the absence or presence of the indicated amount of ASA-LDL or native LDL. Results, expressed in ng ^{125}I-LDL/mg cell proteins, have been converted into percentages: 100% = 125 ± 26 ng LDL bound/mg; 646 ± 76 ng LDL internalized/mg and 1247 ± 182 ng LDL degraded/mg. Means of 3 experimental values.

human fibroblasts (fig.2a–c) indicated that ASA-LDL is less effective than native LDL in inhibiting ^{125}I-LDL binding, uptake and degradation. The fact that the decreases in LDL binding, uptake and degradation were of the same order of magnitude suggests that decreased uptake and degradation

Table 1

Competition studies with ASA-LDL and native LDL on ^{125}I-AC-LDL uptake and degradation by the macrophage cell line J774

Addition	Binding	Uptake	Degradation
None	80 ± 15 (100%)	642 ± 75 (100%)	956 ± 114 (100%)
AC-LDL	34 ± 4 (43%)	257 ± 31 (40%)	335 ± 31 (35%)
ASA-LDL	78 ± 11 (98%)	625 ± 66 (97%)	967 ± 109 (101%)

^{125}I-LDL uptake and degradation were determined using 10 µg/ml ^{125}I-AC-LDL in the absence or presence of 10 µg/ml unlabeled AC-LDL or ASA-LDL. Results are expressed as ng ^{125}I-AC-LDL/mg protein. Means of 3 determinations ± SD

were most likely secondary to the decrease in LDL binding. Thus, the ability of ASA-LDL to bind to the apo B/E receptor of fibroblasts was reduced, and this is probably due to the increase in negative net charge of LDL by ASA modification. To determine whether ASA-LDL is recognized by the scavenger receptor of macrophages, we used the mouse macrophage cell line J774 for competition studies with ^{125}I-AC-LDL. The data in table 1 show that ASA-LDL did not compete significantly with AC-LDL. This suggests that LDL modification by ASA did not induce sufficient alterations in the particle to allow its recognition by the AC-LDL receptor of macrophages.

As is the case for other serum proteins, ASA probably induces acetylation of some lysine residues of apolipoprotein B, as suggested by the decrease in free amino groups observed with the TNBS assay. Previous studies have demonstrated a relationship between the number of modified lysine residues and the decreased affinity for the apo B/E receptor of fibroblasts [5]. In the case of ASA-LDL, a discrete alteration of the LDL particle (less than 10% of the lysine residues), undetectable by immunodiffusion studies, is sufficient to induce a significant alteration of the recognition by the fibroblast LDL receptor. However, this modification is insufficient to allow binding to the scavenger receptor of macrophages.

It should be noted that in the experiments described, the dose of ASA used was close to those used therapeutically [2]. Thus, similar alterations in LDL structure and metabolism may occur in vivo in patients treated with ASA. As ASA is widely utilized, the possibility of perturbations in LDL catabolism by the apo B/E receptor pathway should be taken into account, especially in long-term treatment. Finally, it can be noted that binding studies provide a sensitive test for the detection of weak alterations in LDL particles.

REFERENCES

[1] Pinckard, R.N., Hawkins, D. and Farr, R.S. (1968) Nature 219, 68–69.
[2] Hawkins, D., Pinckard, R.N., Crawford, I.P. and Farr, R.S. (1969) J. Clin. Invest. 48, 536–542.
[3] Hawkins, D., Pinckard, R.N. and Farr, R.S. (1968) Science 160, 780–781.
[4] Weisgraber, K.H., Innerarity, T.L. and Mahley, R.W. (1978) J. Biol. Chem. 253, 9053–9062.
[5] Brown, M.S., Goldstein, J.L., Krieger, M., Ho, Y.K. and Anderson, R.G.W. (1979) J. Cell Biol. 82, 597–613.
[6] Mahley, R.W., Innerarity, T.L., Weisgraber, K.H. and Ho, Y.K. (1979) J. Clin. Invest. 64, 743–750.
[7] Haberland, M.E., Fogelman, A.M. and Edwards, P.A. (1982) Proc. Natl. Acad. Sci. USA 79, 1712–1716.
[8] Havel, R.J., Eder, H.A. and Bragdon, J.H. (1955) J. Clin. Invest. 34, 1345–1353.
[9] Bilheimer, D.W., Eisenberg, S. and Levy, R.I. (1972) Biochim. Biophys. Acta 260, 212–221.
[10] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[11] Basu, S.K., Goldstein, J.L., Anderson, R.G.W. and Brown, M.S. (1976) Proc. Natl. Acad. Sci. USA 73, 3178–3182.
[12] Habeeb, A.F.S.A. (1966) Ann. Biochem. 14, 328–336.
[13] Ouchterlony, O. (1964) in: Immunological Methods (Ackroyd, J.F. ed.) pp.5–91, Blackwell, London.
[14] Mazière, J.C., Mazière, C., Mora, L., Auclair, S., Goldstein, S. and Polonovski, J. (1986) FEBS Lett. 195, 135–139.